FILE 'CAPLUS' ENTERED AT 10:53:39 ON 17 FEB 2006

=> S PET11D;S PET22B
L1 25 PET11D

L2 68 PET22B

=> S L1 OR L2
L3 91 L1 OR L2

=> D 1-91 TI

=> D 7,12,13,22,26,31,42,43,46,49,52,54-56,60,61,70-74,79-81,89 CBIB ABS

L3 ANSWER 7 OF 91 CAPLUS COPYRIGHT 2006 ACS on STN
2005:811851 Document No. 144:81788 Molecular cloning of a phytase gene (phy
M) from Pseudomonas syringae MOK1. Cho, Jaiesoon; Lee, Changwhan; Kang, Seungha; Lee, Jaecheon; Lee, Honggu; Bok, Jinduck; Woo, Junghee; Moon, Yangsoo; Choi, Yunjaie (Inositide Signaling Group, National Institute of Environmental Health Sciences, DHSS, NIH, Research Triangle Park, NC, 27709, USA). Current Microbiology, 51(1), 11-15 (English) 2005. CODEN: CUMIDD. ISSN: 0343-8651. Publisher: Springer Science+Business Media, Inc..

AB A phytase gene (phyM) was cloned from Pseudomonas syringae MOK1 by two steps of degenerate PCR and inverse PCR. This gene consists of 1287 nucleotides and encodes a polypeptide of 428 amino acids with a deduced mol. mass of 46,652 kDa. Based on its amino acid sequence, the PhyM shares the active site RHGXRXP and HD sequence motifs, typically characterized by histidine acid phosphatases family. Each phyM gene fragment encoding mature PhyM with its own signal sequence (pEPSS) and without (pEPSM) was subcloned into the E. coli BL21 (DE3) expression vector, **pET22b** (+). The enzyme activity in crude exts. of clone pEPSM was 2.514 Umg-1 of protein, and about 10-fold higher than that of clone pEPSS.

L3 ANSWER 12 OF 91 CAPLUS COPYRIGHT 2006 ACS on STN
2005:559702 Document No. 143:299895 Introducing tandem of transcription terminators increases the efficiency of accumulation of recombinant proteins in Escherichia coli cells when the expression system on the basis
of T7 phage RNA-polymerase is used. Gavrikov, A. V.; Zimenkov, D. V.; Mashko, S. V. (Closed Joint Stock Venture Mosagrogen, Moscow, 117545, Russia). Biotekhnologiya (2), 18-25 (Russian) 2005. CODEN: BTKNEZ. ISSN: 0234-2758. Publisher: Biotekhnologiya.

AB The substitution of the infrequent in Escherichia coli eucaryotic amino acid codons by the bacterial analogs in the structural portion of hIFN-αF gene has been performed. After that, the gene was expressed in E. coli cells with the help of phage T7 RNA-polymerase. It was shown that the accumulation of the target protein can be strongly enhanced by introduction of a tandem of T.vphi. terminators (in-stead of one terminator that is widely used in com. available vectors) in the 3'-non-translated region of the appropriate gene. The percentage of the recombinant hIFN protein in-creased from 10% to more than 20% of the total cell protein 2 h after the induction of the expression system. It

seems unlikely that addnl. stabilizing of 3'-region of mRNA or preventing of the neg. interference between the efficient gene transcription and recombinant plasmid replication that underlies the effect of introduction of terminator tandem on the synthesis of the recombinant protein. More probably, this effect is due to saving the NTP pool and reorientation of T7 RNA-polymerase at the target mRNA synthesis of the cloned gene rather than cyclic transcription of a recombinant plasmid.

L3 ANSWER 13 OF 91 CAPLUS COPYRIGHT 2006 ACS on STN

2005:539558 Document No. 144:123685 Sequence analysis, cloning and over-expression of an endoxylanase from the alkaliphilic Bacillus halodurans. Martinez, M. Alejandra; Delgado, Osvaldo D.; Baigori, Mario D.; Sineriz, Faustino (Planta Piloto de Procesos Industriales Microbiologicos, PROIMI, Tucuman, T-400 1MVB, Argent.). Biotechnology Letters, 27(8), 545-550 (English) 2005. CODEN: BILED3. ISSN: 0141-5492.

Publisher: Springer.

AB The BhMIR32 xyn11A gene, encoding an extracellular endoxylanase of potential interest in bio-bleaching applications, was amplified from Bacillus halodurans MIR32 genomic DNA. The protein encoded is an endo-1,4-β-xylanase belonging to family 11 of glycosyl hydrolases. Its nucleotide sequence was analyzed and the mature peptide was subcloned into **pET22b**(+) expression vector. The enzyme was over-expressed in a high d. Escherichia coli culture as a soluble and active protein, and purified in a single step by immobilized metal ion affinity chromatog. with a specific activity of 3073 IU mg-1.

L3 ANSWER 22 OF 91 CAPLUS COPYRIGHT 2006 ACS on STN

2005:59900 Document No. 142:128718 Preparation of ribonucleases with transgenic cells for use in cancer treatment. Saxena, Shailendra K. (Alfacell Corporation, USA). U.S. Pat. Appl. Publ. US 2005014161 A1 20050120, 32 pp. (English). CODEN: USXXCO. APPLICATION: US 2003-621741 20030717.

AB Methods for recombinantly producing new RNases, as well as previously-known RNases, are disclosed. The new RNases are active against human carcinoma cells. Thus, for each RNase gene, 14 oligonucleotides were synthesized and ligated. This full-length gene was then amplified by PCR to incorporate XbaI and BamHI restriction sites at the termini so that the gene could be ligated into plasmid pET-11d. The primers also introduced a translation initiation codon and a translation termination codon. E. coli was transformed with the recombinant plasmid and cultured to produce the RNase. The RNase was produced with an N-terminal methionine. This was removed with Aeromonas aminopeptidase.

L3 ANSWER 26 OF 91 CAPLUS COPYRIGHT 2006 ACS on STN

2004:950159 Document No. 142:128701 Process for producing β-secretase. Jang, Chang Hwan; Mook, In Hui (Digital Biotech Co., Ltd., S. Korea). Repub. Korean Kongkae Taeho Kongbo KR 2002002188 A 20020109, No pp. given (Korean). CODEN: KRXXA7. APPLICATION: KR 2000-58300 20001004. PRIORITY:

KR 2000-35841 20000628.

AB A process for producing β-secretase is provided, thereby mass-producing the β-secretase which is necessary for the study on Alzheimer's disease. The process for producing β-secretase comprises the steps of: selectively amplifying cDNA of β-secretase and inserting the β-secretase gene into a vector to produce a recombinant expression vector; transforming a host cell with the recombinant expression vector; cultivating the transformed host cell; and separating and purifying the β-secretase produced, in which the β-secretase gene is normal or mutant gene (D215N), wherein 215th amino acid D (Asp) is substituted by N (Asn); the transformed host cell can be E. coli BL21(DE3)/pET22b-BACE22501 (KCTC 18031P), E. coli BL21(DE3)/pET19b-MBACE46419 (KCTC 18040P), and mammal HEK293 CELL/pcDNA3-BACE (KCLRF-BP-00031).

L3 ANSWER 31 OF 91 CAPLUS COPYRIGHT 2006 ACS on STN

2004:533812 Document No. 141:66261 Recombinant autocyclized and cysteinized ranpirnase containing cleavable PelB leader peptide and Met23→L and Ser72→Cys mutations, its cDNA and expression vector, and methods of making them. Saxena, Shailendra K. (Alfacell Corporation, USA). U.S. Pat. Appl. Publ. US 2004126865 A1 20040701, 11 pp. (English). CODEN: USXXCO. APPLICATION: US 2002-331910 20021230.

AB The present invention relates to recombinant ranpirnase (RNase of Rana pipiens) with first Met removed and Met23→L and Ser72→Cys mutations, and its expression vector. RNase DNA coding for an amino acid sequence beginning with a residue of glutamine is introduced into a vector of pET22b(+) plasmid to form recombinant plasmid DNA that begins with a PelB leader sequence. The encoded protein without N-terminal methionine residue can be made by cleaving PelB signal peptide in E. coli host cells and allowing the resulting first glutamine residue to autocyclize to pyroglutamic acid. Other mutations in the recombinant RNase include Met23→L and Ser72→Cys mutations.

L3 ANSWER 42 OF 91 CAPLUS COPYRIGHT 2006 ACS on STN

2002:491090 Document No. 137:243034 Method of presentation of a polypeptide from Escherichia coli using the carboxypeptidase Y propeptide as a fusion partner and a recombinant vector for transformation. Park, Jin Gyu; Oh, Gwi Hwan; Jung, Bong Hyeon; Ham, Mun Seon (Dong Kook Pharm. Co., Ltd., S. Korea; Korea Institute of Science and Technology). Repub. Korean Kongkae Taeho Kongbo KR 2000066750 A 20001115, No pp. given (Korean). CODEN: KRXXA7. APPLICATION: KR 1999-14065 19990420.

AB A novel fusion partner, the propeptide of carboxypeptidase Y (CPY), for effective representation of polypeptide in Escherichia coli, a recombinant vector, and its transformed cell are provided, by which small polypeptides (that are easily degradable in E. coli) can be stably produced. The process comprises: separating genomic DNA of Saccharomyces cerevisiae; processing PCR using the separated genomic DNA as a template with a synthetic primer; separating the PCR fraction from an agarose gel (fraction 1: 287 bp) with restriction enzymes NdeI and

XhoI; insertion into **pET22b+** vector; transformation of E. coli BL21(DE3) for presentation; and inoculating the transformed E. coli and non-transformed E. coli to LB (yeast extract 0.5%, Tryptone 1%, NaCl 1%) liquid medium containing ampicillin, and confirming representation by adding IPTG (isopropyl-β-D- thiogalactopyranoside) while culturing at 37°. The CPY propeptide exists in the form of a non-soluble precipitated body, but is easily dissolved in urea.

L3 ANSWER 43 OF 91 CAPLUS COPYRIGHT 2006 ACS on STN
2002:402637 Document No. 137:197506 Co-expression of caiB and caiE with incompatible plasmids in E. coli. Fan, Liqiang; Yuan, Qinsheng; Wu, Xiangfu (State Key Laboratory of Bioreactor Engineering and Institute of Biochemistry, East China University of Science and Technology, Shanghai, 200237, Peop. Rep. China). Zhongguo Yiyao Gongye Zazhi, 33(3), 113-116 (Chinese) 2002. CODEN: ZYGZEA. ISSN: 1001-8255. Publisher: Zhongguo Yiyao Gongye Zazhi Bianjibu.

AB A recombinant bacterium exhibiting high enzymic activity was obtained by cloning and coexpression of both caiB and caiE genes in a host of E. coli BL21 (DE3), which encoded carnitine dehydratase and a protein that may be related to synthesis of cofactor for carnitine dehydratase, resp. The genes were isolated from pSK-caiB or pSK-caiE and then were ligated into expression vector pET28a (+) or **pET22b** (+) to construct plasmid pET28caiB or pET22caiE. These two plasmids were stably transformed and maintained in E. coli BL21(DE3) when both ampicillin and kanamycin were presented in the selective medium. After induction with IPTG, both caiB and caiE genes were coexpressed and expressed products accounted for 39% and 20% of the total proteins in the host. Compared with E. coli BL21(DE3) containing only pET28caiB, the activity of carnitine dehydratase increased 2.3-fold in the same strain containing two plasmids. A new method for coexpression of proteins in E. coli containing two incompatible plasmids, in which two different antibiotic resistance markers were included, was also established.

L3 ANSWER 46 OF 91 CAPLUS COPYRIGHT 2006 ACS on STN
2002:318068 Document No. 137:165298 Cloning, high-level expression, purification and crystallization of peptide deformylase from Leptospira interrogans. Li, Yikun; Ren, Shuangxi; Gong, Weimin (School of Life Sciences, University of Science and Technology of China, Hefei, Anhui, 230026, Peop. Rep. China). Acta Crystallographica, Section D: Biological Crystallography, D58(5), 846-848 (English) 2002. CODEN: ABCRE6. ISSN: 0907-4449. Publisher: Blackwell Munksgaard.

AB A new peptide deformylase (PDF; E.C. 3.5.1.27) gene from Leptospira interrogans was identified and cloned into expression plasmid **pET22b**(+) and was highly expressed in Escherichia coli BL21(DE3). With DEAE-Sepharose anion-exchange chromatog. followed by Superdex G-75 size-exclusion chromatog., 60 mg of PDF from L. interrogans was purified from 1 l of cell culture. Crystallization screening of the purified enzyme resulted in two crystal forms, from one of which a 3 Å resolution x-ray diffraction data set has been collected.

L3 ANSWER 49 OF 91 CAPLUS COPYRIGHT 2006 ACS on STN
2002:30719 Document No. 136:243646 Kynurenine aminotransferase and glutamine

transaminase K of Escherichia coli: identity with aspartate aminotransferase. Han, Qian; Fang, Jianmin; Li, Jianyong (Department of Pathobiology, University of Illinois at Champaign-Urbana, Urbana, IL, 61802, USA). Biochemical Journal, 360(3), 617-623 (English) 2001.
CODEN:
BIJOAK. ISSN: 0264-6021. Publisher: Portland Press Ltd..

AB The present study describes the isolation of a protein from Escherichia coli possessing kynurenine aminotransferase (KAT) activity and its identification as aspartate aminotransferase (AspAT). KAT catalyzes the transamination of kynurenine and 3-hydroxykynurenine to kynurenic acid and xanthurenic acid resp., and the enzyme activity can be easily detected in E. coli cells. Separation of the E. coli protein possessing KAT activity through various chromatog. steps led to the isolation of the enzyme. N-terminal sequencing of the purified protein determined its first 10 N-terminal amino acid residues, which were identical with those of the E. coli AspAT. Recombinant AspAT (R-AspAT), homologously expressed in an E. coli/**pET22b** expression system, was capable of catalyzing the transamination of both L-kynurenine (Km = 3 mM; Vmax = 7.9 μmol.min-1.mg-1) and 3-hydroxy-DL-kynurenine (Km = 3.7 mM; Vmax = 1.25 μmol.min-1.mg-1) in the presence of pyruvate as an amino acceptor, and exhibited its maximum activity at temps. between 50-60°C and at a pH of approx. 7.0. Like mammalian KATs, R-AspAT also displayed high glutamine transaminase K activity when L-phenylalanine was used as an amino donor (Km = 8 mM; Vmax = 20.6 μmol.min-1.mg-1). The exact match of the first ten N-terminal amino acid residues of the KAT-active protein with that of AspAT, in conjunction with the high KAT activity of R-AspAT, provides convincing evidence that the identity of the E. coli protein is AspAT.

L3 ANSWER 52 OF 91 CAPLUS COPYRIGHT 2006 ACS on STN
2001:237066 Document No. 135:367422 Enhanced expression of cytochrome P450s
from lac-based plasmids using lactose as the inducer. Woyski, Denice; Cupp-Vickery, Jill R. (Department of Chemistry and Biochemistry, California State University, Fullerton, Fullerton, CA, 92834, USA). Archives of Biochemistry and Biophysics, 388(2), 276-280 (English) 2001.
CODEN: ABBIA4. ISSN: 0003-9861. Publisher: Academic Press.

AB The cytochrome P 450 expression systems used in Escherichia coli are highly regulated and involve the use of the lac repressor to control expression. Induction in these systems utilizes the nonmetabolizable analog of lactose, isopropyl-β-D-thiogalactopyranoside (IPTG), which is the most expensive compound required for an E. coli expression system. To determine if the natural inducer lactose could be used to induce cytochrome P 450 expression the expression of three P 450 enzymes was examined in E. coli using two different expression systems, pTrc99A and the T7-based **PET22b** vector. For both systems lactose was found to induce expression of active P 450 to concns. that exceeded the levels achieved with IPTG. A 20-L fermentation of a P 450 expression system in the pTrc plasmid in which lactose was used as the inducer resulted in 2.4 μ mol P 450/L, with a total yield of 2 g of cytochrome P 450. The use of lactose for protein expression in E. coli should be broadly useful for the inexpensive, large-scale production of heterologous proteins in E. coli. (c) 2001 Academic Press.

L3 ANSWER 54 OF 91 CAPLUS COPYRIGHT 2006 ACS on STN

2001:45196 Document No. 134:112234 Cloning of ranpirnase and its mutant. Saxena, Shailendra K. (Alfacell Corporation, USA). U.S. US 6175003 B1 20010116, 7 pp. (English). CODEN: USXXAM. APPLICATION: US 1999-394268 19990910.

AB A **pET11d**-rOnc(Q1, M23L) DNA is subjected to two different site-directed mutations, each using an overlapping PCR protocol. One of the site-directed mutations changes the amino acid residue at position 23 of the encoded protein from leucine to methionine, whereby the encoded protein can be made into ranpirnase (RNase of Rana pipiens) by cleaving the N-terminal methionine residue and allowing the adjacent glutamine residue to autocyclize. The other site-directed mutation changes the amino acid residue at position 72 of the encoded protein from serine to cysteine, thereby producing an encoded protein that can be made into a cysteinized ranpirnase by cleaving the N-terminal methionine residue and allowing the adjacent glutamine residue to autocyclize.

L3 ANSWER 55 OF 91 CAPLUS COPYRIGHT 2006 ACS on STN

2000:850535 Document No. 134:159265 Overexpression and characterization of Vibrio mimicus metalloprotease. Shin, Seung-Yeol; Lee, Jong-Hee; Huh, Sung-Hoi; Park, Young-Seo; Kim, Jin-Man; Kong, In-Soo (Department of Biotechnology and Bioengineering, Pukyong National University, Pusan, 608-737, S. Korea). Journal of Microbiology and Biotechnology, 10(5), 612-619 (English) 2000. CODEN: JOMBES. ISSN: 1017-7825. Publisher: Korean Society for Applied Microbiology.

AB To investigate the biochem. properties of V. mimicus metalloprotease, whose gene was isolated previously from Vibrio mimicus ATCC33653, overexpression and purification were attempted. The 1.9 kb of open reading frame was amplified by PCR from pVMC193 plasmid which ligated the vmc gene with pUC19 and introduced into Escherichia coli BL21 (DE3) using the overexpression vector, **pET22b** (+). Overexpressed metalloprotease (VMC) was purified with Ni-NTA column chromatog. and characterized with various protease inhibitors, pHs, temps., and substrates. Purified VMC showed proteolytic activity against gelatin, soluble and insol. collagens, and synthetic peptides. Unlike observations made with all metalloproteases originated from other Vibrio sp., VMC did not hydrolyze casein. Proteolytic activity was critically decreased when VMC was treated with metal chelating reagents, such as EDTA, 2,2-bipyridine, and 1,10-phenanthroline. In particular, the 71 kDa VMC exhibited hemagglutinating activity against human erythrocyte. As purified VMC was treated with CuCl2 and NiCl2 for the chemical modification of metal binding, the proteolytic activity and hemagglutinating activity were profoundly influenced. Multialignment anal. made on the reported Vibrio metalloproteases showed the difference of amino acid sequence similarity between two distinctive classes of Vibrio metalloproteases.

L3 ANSWER 56 OF 91 CAPLUS COPYRIGHT 2006 ACS on STN

2000:291182 Document No. 133:55210 His-tagged tryparedoxin peroxidase of Trypanosoma cruzi as a tool for drug screening. Guerrero, S. A.; Lopez, J. A.; Steinert, P.; Montemartini, M.; Kalisz, H. M.; Colli, W.; Singh, M.; Alves, M. J. M.; Flohe, L. (Department of Biochemistry, Technical University of Braunschweig, Braunschweig, D-38124, Germany). Applied Microbiology and Biotechnology, 53(4), 410-414 (English) 2000. CODEN: AMBIDG. ISSN: 0175-7598. Publisher: Springer-Verlag.

AB Tryparedoxin peroxidase has recently been identified as a constituent of the complex peroxidase system in the trypanosomatid Crithidia fasciculata. In trypanosomatids, hydroperoxides are reduced at the expense of NADPH by means of a cascade of three oxidoreductases: the flavoprotein trypanothione reductase, tryparedoxin and tryparedoxin peroxidase. Inhibitors of these enzymes are presumed to be trypanocidal drugs. Here, we present the heterologous expression of a putative tryparedoxin peroxidase gene of Trypanosoma cruzi (accession no AJ012101) as an N-terminally His-tagged protein (TcH6TXNPx). The product was purified with a high yield (8.75 mg from 1 l fermentation broth of A600 2.1) from the cytosolic fraction of sonified Escherichia coli BL21(DE3)[**pET22b** (+)/TcH6TXNPx] by metal-chelating chromatog. TcH6TXNPx proved to be fully active when tested with heterologous tryparedoxins of C. fasciculata (His-tagged TXN1H6 and TXN2H6). TcH6TXNPx displayed ping-pong kinetics with a kcat of 1.7 s-1 and limiting Km values of 51.8 μM and 1.7 μM for t-Bu hydroperoxide and CfTXN2H6, resp.

L3 ANSWER 60 OF 91 CAPLUS COPYRIGHT 2006 ACS on STN

2000:36499 Document No. 132:107085 Construction of an expression system of shikimate kinase II and preparation of shikimic acid 3-phosphate. Akiyama, Hiroshi; Okunuki, Haruyo; Tsuzuki, Satoko; Arami, Shinichiro; Miura, Hirohito; Sakushima, Junichiro; Teshima, Reiko; Goda, Yukihiro; Hino, Akihiro; Sawada, Junichi; Toyoda, Masatake (Natl. Inst. of Health Sci., 1-18-1, Kamiyoga, Setagaya-ku, Tokyo, 158-8501, Japan). Shokuhin Eiseigaku Zasshi, 40(6), 438-443 (Japanese) 1999. CODEN: SKEZAP. ISSN: 0015-6426. Publisher: Nippon Shokuhin Eisei Gakkai.

AB Shikimic acid 3-phosphate (S-3-P) is needed as a substrate to detect the enzyme activity of 5-enolpyruvylshikimic acid 3-phosphate synthase (CP4EPSPS), which is expressed in genetically modified soybeans. Therefore, we attempted the over-expression fo shikimate kinase II (SK-II) in E. coli to biosynthetically obtain S-3-P. The aroL gene encoding SK-II was constructed in the expression vector **pET22b**(+). The aroL expression vector was then transfected into E. coli strain BL21 using the electroporation method. The activity of the obtained aroL protein was confirmed by HPLC using an amino-silica column and incorporation of [32P] from labeled ATP to shikimic acid. The determination of the reaction product was performed by LC/MS using a carbon column and periodate treatment. HPLC using a carbon column does not use a non-volatile buffer as the mobile phase. Thus, this method should be useful for preparing S-3-P from the crude reaction mixture of SK-II.

L3 ANSWER 61 OF 91 CAPLUS COPYRIGHT 2006 ACS on STN

1999:810732 Document No. 132:121511 Overexpression of cyclodextrin glycosyltransferase gene from Brevibacillus brevis in Escherichia coli by control of temperature and mannitol concentration. Kim, Myung Hee; Lee, Jung Kee; Kim, Hyung Kwoun; Sohn, Cheon Bae; Oh, Tae Kwang (Microbial Enzyme Research Unit, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Taejon, 305-600, S. Korea). Biotechnology Techniques, 13(11), 765-770 (English) 1999. CODEN: BTECE6. ISSN: 0951-208X. Publisher: Kluwer Academic Publishers.

AB Expression of Brevibacillus brevis CD162 cyclodextrin glycosyltransferase (CGTase) gene using **pET22b**(+) vector in Escherichia coli BL21(DE3) resulted in the formation of inactive inclusion bodies

under the usual induction conditions. However, by lowering the induction temperature to 30°C and/or adding 0.5 M mannitol as an osmolyte, the formation of insol. aggregates was prevented and about a 34-fold increase (8.51 U ml-1) in biol. active soluble form was achieved after 6 h induction. The active CGTase enzyme was estimated to comprise as much as 24% of the total soluble proteins. In addition, other polyols such as glycerol, erythritol, xylitol, sorbitol, and arabitol showed similar effects with mannitol on the production of active CGTase enzyme.

L3 ANSWER 70 OF 91 CAPLUS COPYRIGHT 2006 ACS on STN

1998:639316 Document No. 130:48093 Cloning and identification of a phospholipase gene from Vibrio mimicus. Kang, Jung-Hwa; Lee, Jong-Hee; Park, Je-Hyeon; Huh, Sung-Hoi; Kong, In-Soo (Department of Biotechnology and Bioengineering, Pukyong National University, Pusan, 608-737, S. Korea). Biochimica et Biophysica Acta, Lipids and Lipid Metabolism, 1394(1), 85-89 (English) 1998. CODEN: BBLLA6. ISSN: 0005-2760. Publisher: Elsevier B.V..

AB The phospholipase gene phl was identified from Vibrio mimicus (ATCC33653) and sequenced. The entire open reading frame (ORF) was composed of 1410 nucleotides and encoding 470 amino acids. The phl was placed upstream of hemolysin gene (vmhA) with opposite direction of transcription. From the BLAST search program, the deduced amino acids sequence showed 74.4% identity with phospholipase gene (lec) from V. cholerae El Tor. The entire ORF of phospholipase gene was amplified by PCR and inserted into an Escherichia coli expression vector, **pET22b(+)** and introduced E. coli BL21(DE3). SDS-PAGE demonstrated that a protein corresponding to the phospholipase was overexpressed and migrated at a mol. mass of 53 kDa.

L3 ANSWER 71 OF 91 CAPLUS COPYRIGHT 2006 ACS on STN

1998:542180 Document No. 129:227358 Expression, purification, and characterization of recombinant Escherichia coli pyridoxine 5'-phosphate oxidase. Di Salvo, Martino; Yang, Emily; Zhao, Genshi; Winkler, Malcolm E.; Schirch, Verne (Department of Biochemistry and Molecular Biophysics, Virginia Commonwealth University, Richmond, VA, 23298, USA). Protein Expression and Purification, 13(3), 349-356 (English) 1998. CODEN: PEXPEJ. ISSN: 1046-5928. Publisher: Academic Press.

AB A previously cloned pdxH gene from E. coli coding for pyridoxine 5'-phosphate oxidase (I) was transferred to a **pET22b** vector and expressed in E. coli HMS174(DE3) cells. The soluble overexpressed enzyme was rapidly purified in high yield using 2 chromatog. columns with an overall purification of .apprx.2.8-fold. Purified I contained tightly bound FMN. I exhibited the same spectral properties and similar kinetic consts. to those previously reported by G. Zhao and M. E. Winkler (1995), but differed from the properties reported by other investigators. A rapid procedure was developed for preparing apo-I in high yield. Both the holo- and apoenzymes were homodimers. The molar absorptivity coefficient for the protein was determined for the fully active I from its amino acid composition Using this value and the spectral properties of the bound FMN, it was shown by 3 different methods that the dimeric enzyme contains 2 mols. of bound FMN per dimer and not 1 FMN as previously reported. (c) 1998 Academic Press.

L3 ANSWER 72 OF 91 CAPLUS COPYRIGHT 2006 ACS on STN
1998:537350 Document No. 129:213335 Purification and characterization of recombinant rabbit cytosolic serine hydroxymethyltransferase. Di Salvo, Martino L.; Fratte, Sonia Delle; De Biase, Daniela; Bossa, Francesco; Schirch, Verne (Dipartimento di Scienze Biochimiche "A. Rossi Fanielli" and Centro di Biologia Molecolare del Consiglio Nazionale delle Ricerche, Univ. La Sapienza, Rome, Italy). Protein Expression and Purification, 13(2), 177-183 (English) 1998. CODEN: PEXPEJ. ISSN: 1046-5928. Publisher: Academic Press.

AB A rabbit liver cDNA library in phage λgt10 was screened using the coding cDNA for human cytosolic serine hydroxymethyltransferase. A clone of 1754 bp was isolated and the nucleotide sequence showed an open reading frame of 1455 bp, which coded for rabbit cytosolic serine hydroxymethyltransferase and was flanked by 12 bp at the 5' end and 287 bp at the 3' end. The full-length cDNA was then cloned into a **pET22b** vector as a NdeI-EcoRI insert. HMS174(DE3) cells were transformed with this plasmid and, after induction with iso-Pr β-D-thiogalactopyranoside, expressed a catalytically active serine hydroxymethyltransferase. The enzyme was purified and shown to be the expressed rabbit enzyme lacking the first methionine residue. Spectral characteristics of the bound pyridoxal phosphate and kinetic consts. for the natural substrates L-serine and tetrahydrofolatel were essentially identical to the values obtained previously for the rabbit cytosolic enzyme. The pattern of bands shown by the pure recombinant enzyme on an isoelec. focusing gel containing 6 M urea showed a major band and a minor band representing about 15-20% of the protein. Upon incubation of the recombinant enzyme at pH 7.3 and 37°C, three new bands were observed on isoelec. focusing with the concomitant formation of isoaspartyl residues, as determined by reactivity with protein isoaspartyl methyltransferase. These results are consistent with deamidation of Asn residues to isoaspartyl during the in vitro incubation. The enzyme purified from rabbit liver has previously been shown to contain isoaspartyl residues. (c) 1998 Academic Press.

L3 ANSWER 73 OF 91 CAPLUS COPYRIGHT 2006 ACS on STN
1998:252654 Document No. 129:63766 Cloning of the thermostable phytase gene (phy) from Bacillus sp. DS11 and its overexpression in Escherichia coli. Kim, Young-Ok; Lee, Jung-Kee; Kim, Hyung-Kwoun; Yu, Ju-Hyun; Oh, Tae-Kwang (Microbial Enzyme RU, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Taejon, 305-600, S. Korea). FEMS Microbiology Letters, 162(1), 185-191 (English) 1998. CODEN: FMLED7. ISSN: 0378-1097. Publisher: Elsevier Science B.V..

AB Phytase hydrolyzes phytate to release inorg. phosphate, which would decrease the addition of phosphorus to feedstuffs for monogastric animals and thus reduce environmental pollution. The gene encoding phytase from Bacillus sp. DS11 was cloned in Escherichia coli and its sequence determined A 560-bp DNA fragment was used as a probe to screen the genomic library. It was obtained through PCR of Bacillus sp. DS11 chromosomal DNA and two oligonucleotide primers based on N-terminal amino acid sequences of the purified protein and the cyanogen bromide-cleaved 21-kDa fragment. The phy cloned was encoded by a 2.2-kb

fragment. This gene comprises 1152 nucleotides and encodes a polypeptide of 383 amino acids with a deduced mol. mass of 41808 Da. Phytase was produced to 20% content of total soluble proteins in E. coli BL21 (DE3) using the **pET22b**(+) vector with the inducible T7 promoter. This is the first nucleic sequence report on phytase from a bacterial strain.

L3 ANSWER 74 OF 91 CAPLUS COPYRIGHT 2006 ACS on STN
1998:212648 Document No. 129:64802 The Rana catesbeiana rcr gene encoding a cytotoxic ribonuclease. Tissue distribution, cloning, purification, cytotoxicity, and active residues for RNase activity. Huang, Huey-Chung; Wang, Sui-Chi; Leu, Ying-Jen; Lu, Shao-Chun; Liao, You-Di (Institute of Biomedical Sciences, Academia Sinica, Taipei, 11529, Taiwan). Journal of Biological Chemistry, 273(11), 6395-6401 (English) 1998. CODEN: JBCHA3. ISSN: 0021-9258. Publisher: American Society for Biochemistry and Molecular Biology.

AB Rana catesbeiana RNase (RC-RNase) is a pyrimidine-guanine sequence-specific RNase found in R. catesbeiana (bullfrog) oocytes. It possesses both RNase activity and cytotoxicity against tumor cells. We report here for the first time the cloning of RC-RNase cDNA from liver rather than from oocytes where RC-RNase is stored. An internal fragment of cDNA was obtained by reverse transcription-PCR using deduced oligonucleotides as primers. Full-length cDNA was obtained by 5'- and 3'-RACE technique. The cDNA clone, named rcr gene, contained a 5'-untranslated region, a putative signal peptide (22 amino acids), a mature protein (111 amino acids), a 3'-untranslated region, and a polyadenylation site. The cDNA which encoded the mature protein was fused upstream with a modified pelB signal peptide DNA and inserted into **pET11d** for expression in Escherichia coli strain BL21 (DE3). The secretory RC-RNase in the culture medium was enzymically active and was purified to homogeneity. The recombinant RC-RNase had the same amino acid sequence, specific activity, substrate specificity, antigenicity, and cytotoxicity as that of native RC-RNase from frog oocytes. Amino acid residues His-10, Lys-35, and His-103 are involved in RC-RNase catalytic activity. Ribonucleolytic activity was involved in and may be essential for RC-RNase cytotoxicity. DNA sequence anal. showed that RC-RNase had approx. 45% identity to that of RNase superfamily genes. This indicates that RC-RNase is a distinct RNase gene in the RNase superfamily.

L3 ANSWER 79 OF 91 CAPLUS COPYRIGHT 2006 ACS on STN
1996:397694 Document No. 125:52098 Purification and characterization of a lentil seedling lipoxygenase expressed in E. coli: implications for the mechanism of oxodiene formation by lipoxygenases. Hilbers, Martin P.; Finazzi-Agro, Alessandro; Veldink, Gerrit A.; Vliegenthart, Johannes F. G. (Dep. Experimental Med. Biochem. Sci., Univ. Rome "Tor Vergata", Rome, I-00135, Italy). International Journal of Biochemistry & Cell Biology, 28(7), 751-760 (English) 1996. CODEN: IJBBFU. ISSN: 1357-2725. Publisher: Elsevier.

AB Lentil seedlings contain at least 6 lipoxygenase isoenzymes, which are difficult to sep. by classical enzyme purification techniques. The aim

of this work was to study one particular lentil seedling lipoxygenase, as previous work indicated possible interesting characteristics of this enzyme with respect to oxodiene formation. Since it proved to be difficult to obtain this enzyme in significant quantities in a pure state, the authors expressed it in Escherichia coli. Using an expression vector based on the phage T7 RNA polymerase promoter (**pET11d**), a fully functional lentil seedling recombinant lipoxygenase was expressed in E. coli and purified to homogeneity by DEAE-Sepharose ion-exchange chromatog. and gel-filtration. The products obtained from linoleic acid were analyzed. This recombinant lipoxygenase corresponded to that found in the lower part of the epicotyl and in the hypocotyl of the lentil seedling. It produced predominantly 13(S)-hydroperoxy-(9Z,11E)-octadecadienoic and minor amts. of 9(S)-hydroperoxy-(10E,12Z)-octadecadienoic acids, as well as significant amts. of C18-oxodienes with a regiospecificity different from hydroperoxide formation. The latter mixture was found to consist of equal amts. of 13-oxo-(9Z,11E)-octadecadienoic and 9-oxo-(10E,12Z)- octadecadienoic acids. It was concluded that (1) oxodienes formed by this lentil enzyme do not originate from a secondary conversion of hydroperoxides, but rather from a different lipoxygenase-catalyzed reaction and (2) this lipoxygenase shows similarities to pea lipoxygenase g, with both representing a novel type of legume lipoxygenase.

L3 ANSWER 80 OF 91 CAPLUS COPYRIGHT 2006 ACS on STN
1996:290379 Document No. 124:309580 Preparation of picornavirus L proteinase
with recombinant bacteria and method for identifying L proteinase inhibitors. Skern, Timothy; Kirchweger, Regina; Ziegler, Elisabeth; Blaas, Dieter; Liebig, hans-Dieter; Lamphear, Barry; Waters, Debora; Rhoads, Robert; Sommergruber, Wolfgang; Ahorn, Horst (Boehringer Ingelheim
Vetmedica Gmbh, Germany). PCT Int. Appl. WO 9603514 A1 19960208, 67 pp.
DESIGNATED STATES: W: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK,
EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1995-EP2742 19950713. PRIORITY: US 1994-279152 19940722.

AB Methods of making and using Picornavirus (foot-and-mouth disease virus) L proteinase peptides (PLPPs) are provided. Nucleotides 892-1896 of foot-and-mouth disease virus were inserted into plasmid **pET11d**. Thus, the T7 RNA polymerase promoter is fused to the viral nucleic acid encoding the Lb form of the leader proteinase, the VP4 protein, and part of the VP2 protein followed by two stop codons. The resulting construct was expressed in Escherichia coli. Due to constitutive expression of the lac repressor from the vector and due to an overlap of the lac operator on the T7 RNA polymerase promoter, efficient repression of the uninduced expression vector was achieved and toxicity of the proteinase was avoided. The primary cleavage site of eIF-4γ by Lb proteinase was determined

L3 ANSWER 81 OF 91 CAPLUS COPYRIGHT 2006 ACS on STN
1996:75320 Document No. 124:137032 High level expression in Escherichia

coli, purification and properties of chloroplast fructose-1,6-bisphosphatase from rapeseed (Brassica napus) leaves. Rodriguez-Suarez, Roberto J.; Wolosiuk, Ricardo A. (Inst. Invest. Bioquim., IIBBA, Buenos Aires, 1405, Argent.). Photosynthesis Research, 46(1-2), 313-22 (English)
1995. CODEN: PHRSDI. ISSN: 0166-8595. Publisher: Kluwer.

AB In chloroplasts, the light-modulated fructose-1,6-bisphosphatase catalyzes the formation of fructose 6-bisphosphate for the photosynthetic assimilation of CO2 and the biosynthesis of starch. We report here the construction of a plasmid for the production of chloroplast fructose-1,6-bisphosphatase in a bacterial system and the subsequent purification to homogeneity of the genetically engineered enzyme. To this end, a DNA sequence that coded for chloroplast fructose-1,6-bisphosphatase of rapeseed (Brassica napus) leaves was successively amplified by PCR, ligated into the NdeI/EcoRI restriction site of the expression vector **pET22b**, and introduced into Escherichia coli cells. When gene expression was induced by isopropyl-β-D-thiogalactopyranoside, supernatants of cell lysates were extremely active in the hydrolysis of fructose 1,6-bisphosphate. Partitioning bacterial soluble proteins by ammonium sulfate followed by anion exchange chromatog. yielded 10 mg of homogeneous enzyme per 1 of culture. Congruent with a preparation devoid of contaminating proteins, the Edman degradation evinced an unique N-terminal amino acid sequence [A-V-A-A-D-A-T-A-E-T-K-P-]. Gel filtration expts. and sodium dodecyl sulfate-polyacrylamide gel electrophoresis indicated that the (recombinant) rapeseed chloroplast fructose-1,6-bisphosphatase was a tetramer [160 kDa] comprised of four identical subunits. Like other chloroplast fructose-1,6-bisphosphatases, the recombinant enzyme was inactive at 1 mM fructose 1,6-bisphosphate and 1 mM Mg2+ but became fully active after an incubation in the presence of either 10 mM dithiothreitol or 1 mM dithiothreitol and chloroplast thioredoxin. However, at variance with counterparts isolated from higher plant leaves, the low activity observed in absence of reductants was not greatly enhanced by high concns. of fructose 1,6-bisphosphate (3 mM) and Mg2∞+ (10 mM). In the catalytic process, all chloroplast fructose-1,6-bisphosphatases had identical features; viz., the requirement of Mg2+ as cofactor and the inhibition by Ca2+. Thus, the procedure described here should prove useful for the structural and kinetic anal. of rapeseed chloroplast fructose-1,6-bisphosphatase in view that this enzyme was not isolated from leaves.

L3 ANSWER 89 OF 91 CAPLUS COPYRIGHT 2006 ACS on STN

1993:554616 Document No. 119:154616 High expression in Escherichia coli of the gene coding for dihydrofolate reductase of the extremely halophilic archaebacterium Haloferax volcanii. Reconstitution of the active enzyme and mutation studies. Blecher, Oshra; Goldman, Sarah; Mevarech, Moshe (George S. Wise Fac. Life Sci., Tel Aviv Univ., Israel). European Journal of Biochemistry, 216(1), 199-203 (English) 1993. CODEN: EJBCAI. ISSN: 0014-2956.

AB The gene coding for the enzyme dihydrofolate reductase of the extremely halophilic archaebacterium H. volcanii was recombined into the Escherichia coli expression vector **pET11d**. Following induction, the enzyme was produced in large quantities and accumulated in the cells in an insol. form. The enzymic activity could be efficiently reconstituted

by dissolving the aggregate in 6M guanidine hydrochloride followed by dilution into salt solns. Mutants were produced in which Lys30 was converted to Leu (K30L), Lys31 was converted to Ala (K31A) and a double mutant in which both lysines were converted (K30L, K31A). The mutated enzymes were produced in E. coli, activated and purified to homogeneity. The effect of the salt concentration on the steady-state kinetic parameters was determined It was found that the salt concentration affects the Km but not kcat of the various mutants.

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=> E SHAILENDRA/AU
=> S E4,E5
             1 "SHAILENDRA N S"/AU
             1 "SHAILENDRA S"/AU
L4           2 ("SHAILENDRA N S"/AU OR "SHAILENDRA S"/AU)

=> S L4 NOT L3
L5           2 L4 NOT L3

=> D 1-2 CBIB ABS
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L5 ANSWER 1 OF 2 CAPLUS COPYRIGHT 2006 ACS on STN
2001:746570 Document No. 136:69943 Synthesis, characterization and antiamoebic activity of new thiophene-2-carboxaldehyde thiosemicarbazone derivatives and their cyclooctadiene Ru(II) complexes. **Shailendra, N. S.**; Bharti, N.; Gonzalez Garza, M. T.; Cruz-Vega, D. E.; Castro Garza, J.; Saleem, K.; Naqvi, F.; Azam, A. (Department of Chemistry, Jamia Millia Islamia, Jamia Nagar, New Delhi, 110025, India). Bioorganic & Medicinal Chemistry Letters, 11(20), 2675-2678 (English) 2001. CODEN: BMCLE8. ISSN: 0960-894X. OTHER SOURCES: CASREACT 136:69943. Publisher: Elsevier Science Ltd..

AB Reaction of new thiosemicarbazones, e.g. C4H4SCH:NNHC(S)NHC5H9, derived from thiophene-2-carboxaldehyde and cycloalkylaminothiocarbonylhydrazine, with [Ru(η4-C8H12)(CH3CN)2Cl2] leads to form complexes of the type [Ru(η4-C8H12)(TSC)Cl2] (TSC = thiosemicarbazone). All the compds. have been characterized by elemental anal., IR, 1H NMR, electronic spectra and thermogravimetric anal. It is concluded that the thionic sulfur and the azomethine nitrogen atom of the ligands are bonded to the metal ion. In vitro antiamoebic screening against (HK-9) strain of Entamoeba histolytica indicated that the Ru(II) complexes of thiophene-2- carboxaldehyde thiosemicarbazones were found more active than the thiosemicarbazones.

L5 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2006 ACS on STN
1998:49912 Document No. 128:98693 Methylamine induced changes in ovary of mice, Mus musculus (p). Shrivastava, V. K.; Bhagawat, S.; David, C. V.; **Shailendra, S.** (Department of Biosciences, Endocrinology Unit, Barkatullah University, Bhopal, 462 026, India). Chemical & Environmental Research, 4(1 & 2), 135-139 (English) 1995. CODEN: CEREEH. ISSN: 0971-2151. Publisher: Muslim Association for the Advancement of Science.

AB Female mice were exposed with methylamine (MA, 50 ppm/day) through drinking water for period of 3 wk and their estrus cycle and ovarian histol. were studied. The MA exposed mice showed irregular estrus cycle. In their vaginal smear, the maximum leukocyte cells and few cornified cells were found which suggested that the animals were in late meta estrus/early diestrus phase. Besides this, MA exposed animals also revealed less sudanophilic reactions in the interstitial cells and thecal layers of the ovary when compared with control. However, an increased nos. of atratic follicles and degenerated oocytes were also noticed. Above findings revealed that the MA inhibits the synthesis of total lipid or cholesterol in the ovary which might be inhibiting the synthesis of steroids that results in the atratic changes in the ovarian tissue. The mode of action of MA may be directly on the ovary or it may modulate the gonadotropic level through pituitary by influencing the hypothalamo-hypophys. gonadal axis.